DNA Casework Unit
Procedures for the Extraction of DNA from Hair and Keratinized Tissue

1 Scope

These procedures describe the process for chemical digestion and purification of deoxyribonucleic acid (DNA) from hair or keratinized tissue (i.e., fingernails).

2 Equipment/Materials/Reagents

Equipment/Materials

- General laboratory supplies (e.g., tubes, pipettes, vortex, forceps)
- Magnetic stand
- Stereomicroscope
- Sonicator
- Micro tissue grinder (aka mortar and pestle), if needed

Reagents

- Xylene and/or xylene substitute, if needed
- Terg-a-zyme, powder or 5% solution
- Ethanol (EtOH), absolute
- Qiagen® Buffer ATL
- Dithiothreitol (DTT), 5M solution
- Proteinase K (ProK), 20mg/mL
- Qiagen® Buffer AL
- PrepFiler® Forensic DNA Extraction Kit
  - PrepFiler® Magnetic Particles
  - PrepFiler® Wash Buffers A and B
  - PrepFiler® Elution Buffer (or TE^{-4} Buffer)
- Isopropanol, 70% Water, reagent grade or equivalent
- Sulfuric acid, 4 N, if needed

3 Standards and Controls

At least one extraction control (i.e., reagent blank [RB]) must be processed in parallel with each extraction batch.

For evaluation of the extraction controls, refer to the appropriate interpretation procedure of the *DNA Procedures Manual*.

4 Procedures

Refer to the DNA Procedures Introduction (DNA QA 600) for applicable general precautions and cleaning instructions.

Supplies typically needed for 1 sample and 1 RB (adjust for batches):

EtOH (~1 mL)	Isopropanol (~400 µL)	Do NOT UV
Water (~10mL)	Wash Buffer A (~2 mL)	Magnetic beads
5M DTT (~15 µL)	Wash Buffer B (~1 mL)	ProK
Buffer ATL (~1 mL)	Elution Buffer (~150 µL)	
Buffer AL (~1 mL)		

- 4 - 1.5 mL tubes (sample, RB, final sample extract, final RB extract)
- Ruler, magnetic stand, forceps, scissors, scalpel, tube rack
- p20, p200, p2000 pipettes

4.1 Sample Collection

Except as noted, the following steps are performed in a hood.

A description of the collected sample will be recorded in the notes.

4.1.1 Hair

Any step involving manipulations of difficult hairs may occur outside of hood with aid of stereomicroscope. Reverse action forceps may aid in grasping a hair. **The hood air flow must be off while transferring/handling hair samples.**

4.1.1.1	View hair under stereomicroscope for presence of root tissue or adherent material.	

If sheath material is present, consult an Examiner.

4.1.1.2	Measure hair and record length. Remove ~ 2 cm of hair from root end and place in a tube.	

The sample may be moistened with water to minimize the effects of static.

4.1.2 Fingernail Clipping

4.1.2.1	Place ~ 3 mm x 3 mm of a fingernail in a tube.	

4.2 Sample Cleaning Methods

Each wash procedure may be performed additional times using fresh cleaning solution. The cleaning method(s) used will be recorded in the notes.

Pulse spin, as necessary, throughout procedure to force sample to bottom of tube. A sample may remain in the same tube for each cleaning procedure with the removal of the cleaning liquid(s). In instances with smaller samples, smaller rinse tubes and/or less liquid may be used.

4.2.1 Xylene Wash (Optional)

4.2.1.1	In chemical fume hood, add enough xylene to cover sample. Sonicate at least 20 minutes in chemical fume hood.	

Alternatively, xylene may be added to the collection tube prior to the addition of sample.

4.2.1.2	Remove xylene and appropriately discard waste. Add enough reagent grade water to cover sample and mix.	

As an alternative to the xylene removal, sample may be transferred from xylene tube to a separate water tube with use of tweezers.

4.2.2 Xylene Substitute Wash (Optional)

4.2.2.1	Transfer sample to tube containing enough UV-treated xylene substitute to cover sample. Sonicate at least 20 minutes.	

4.2.3 Terg-a-zyme Wash

To make 5% Terg-a-zyme solution, add 0.5 g Terg-a-zyme to 10 mL water.

4.2.3.1	Transfer sample to tube containing enough 5% Terg-a-zyme solution to cover sample. Sonicate at least 20 minutes.	

4.2.3.2	Transfer sample to tube containing enough EtOH to cover sample and mix to rinse.	

4.2.3.3	Transfer sample to tube containing enough water to cover sample and mix to rinse.	

4.3 Digestion

4.3.1	Enter appropriate barcodes and prepare Digestion Buffer.	

To make Digestion Buffer, add 13.2 µL 5M DTT to 1 mL Buffer ATL.

4.3.2	Add 300 µl of Digestion Buffer and 20µL ProK to each sample and RB tube. Ensure samples are submerged. *Samples may be cut into pieces to ensure full immersion.* Vortex and incubate tubes at 56ºC at 900 rpm for a minimum of ~30 minutes, until the sample is fully digested, or overnight (O/N).	

Sample is generally transferred from the last cleaning tube to a new tube containing the digestion buffer; however, the sample may remain in the same tube with the removal of the water rinse prior to the addition of the digestion buffer.

NOTE: For keratinized tissues, a minimum incubation time of 2 hrs is recommended.

If full digestion does not occur after a minimum of 2 hrs, a partially-digested hair sample (and RB) may undergo the grinding process at the end of this procedure.

4.3.3	Pulse spin. Add 300 µL Buffer AL. Vortex tubes and incubate at 70°C at 900 rpm for 10 minutes.	

4.3.4	Pulse spin and allow to come to room temperature (~5 minutes).	

4.4 PrepFiler® DNA Extraction Kit Purification

Prior to addition, vortex PrepFiler® Magnetic Particles tube for 5 seconds until no visible pellet remains in bottom of tube. Pulse spin. *If processing multiple samples, vortex every ~5 minutes.*

4.4.1	Add 15 µL of Magnetic Particles. Vortex at **low speed** for 10 seconds. Pulse spin.	

4.4.2	Add 180 µL of isopropanol. Vortex at **low speed** for 5 seconds. Mix at room temperature at 1,000 rpm for 10 minutes in shaker.	

4.4.3	Vortex at **high speed** for 10 seconds. Pulse spin.	

4.4.4	Place tubes in magnetic stand. Wait until size of pellet on back of tubes stops increasing (~3 minutes).With tubes in magnetic stand, pipette off and discard all liquid without disturbing pellet.	

4.4.5	Add 600 µL Wash Buffer A. Vortex at **high speed** until there is no visible pellet on side of tube (~5 seconds). Pulse spin.	

It is acceptable to have visible aggregates in solution or on side of tube below meniscus.

4.4.6	Place tubes in magnetic stand for ~60 seconds. With tubes in magnetic stand, pipette off and discard all liquid without disturbing pellet.	

4.4.7	Add 300 µL Wash Buffer A. Vortex at **high speed** until there is no visible pellet on side of tube (~5 seconds). Pulse spin.	

It is acceptable to have visible aggregates in solution or on side of tube below meniscus.

4.4.8	Place tubes in magnetic stand for ~60 seconds. With tubes in magnetic stand, pipette off and discard all liquid without disturbing pellet.	

4.4.9	Add 300 µL Wash Buffer B. Vortex at **high speed** until there is no visible pellet on side of tube (~5 seconds). Pulse spin.	

It is acceptable to have visible aggregates in solution or on side of tube below meniscus.

4.4.10	Place tubes in magnetic stand for ~60 seconds. With tubes in magnetic stand, pipette off and discard all liquid without disturbing pellet. With tubes remaining in magnetic stand, open and air-dry in hood with blower for ~8 minutes.	

DO NOT OVERDRY.

If the room temperature is >25°C, reduce the drying time to 5 minutes.

4.4.11	Add 65µL of Elution Buffer or TE^{-4}. Vortex at **high speed** until there is no visible pellet on side of tube (~5 seconds). Pulse spin.	

If an alternate volume is used for elution, record the volume in the case notes.

4.4.12	Incubate at 70°C and 900 rpm for 5 minutes. Vortex at **high speed** until there is no visible pellet on side of tube (~2 seconds). Pulse spin.	

4.4.13	Place tubes in magnetic stand. Wait until size of pellet on back of tubes stops increasing (~2 minutes.). Transfer liquid into final extract tube without disturbing pellet.	

If an extract is discolored, spin 10,000 X g for 7 minutes and transfer supernatant to new tube.

4.5 Grinding (If necessary)

If necessary, the following procedure may be utilized if full digestion has not occurred after a minimum of 2 hrs.

4.5.1	Moisten swab with 5% Terg-a-zyme solution. *Terg-a-zyme may be warmed prior to use.* Scrub mortar and pestle with swab. Simulate grinding. Rinse with water. Repeat two times.	

4.5.2	In chemical fume hood, add 300uL 4 N sulfuric acid to mortar and simulate grinding. Soak mortar and pestle in 4 N sulfuric acid for 20 minutes.	

4.5.3	Rinse the mortar and pestle with water. Pulse spin the pestle. Remove remaining water and crosslink.	

4.5.4	Transfer 200 µl of solution from the RB tube to mortar and simulate grinding. Remove pestle from mortar. Transfer liquid back to RB tube.	

4.5.5	Transfer 200 µl of solution and the undigested sample fragments to mortar and grind until fragments are no longer visible. Remove pestle from mortar. Transfer liquid back to sample tube.	

4.5.6	Add 20µL ProK and 4µL of 5M DTT to each sample and RB tube. Vortex and incubate at 56ºC at 900 rpm for a minimum of 30 minutes and a maximum of O/N. Pulse spin. Resume digestion processing.	

5 Sample Selection

Generally, an individual hair or nail is received for processing and the sample is collected as described above. When multiples hairs or fingernails are to be processed as a known sample, there is a reasonable assumption of homogeneity and no sampling plan is needed.

6 Calculations

Not applicable.

7 Measurement Uncertainty

Not applicable.

8 Limitations

The quantity and quality of the DNA present within any biological material ultimately determines if a DNA extraction is successful.

A hair does that not fully digest may proceed through this procedure without additional grinding.

9 Safety

9.1 All evidence containing or contaminated with blood or other potentially infectious materials will be considered infectious regardless of the perceived status of the source individual or the age of the material. Follow the "Safe Work Practices and Procedures," "Bloodborne Pathogen (BBP) Exposure Control Plan (ECP)," "Personal Protective Equipment Policy," and "Chemical Hygiene Plan" sections of the *FBI Laboratory Safety Manual*.

9.2 Refer to the "Hazardous Waste Disposal" section of the *FBI Laboratory Safety Manual* for important information concerning proper disposal of the chemicals used in these procedures as well as the biohazardous wastes generated.

9.3 Procedural Specific Chemical Hazards:

- Solutions of Proteinase K can be irritating to mucous membranes. Use eye protection when handling.
- Xylene is an irritant and is toxic. Its use should be confined to a chemical fume hood whenever possible.
- Sulfuric acid is caustic. Gloves, safety glasses, and a laboratory coat must be worn whenever using sulfuric acid. Addition of sulfuric acid to the grinder must be performed inside a chemical fume hood.

10 References

FBI Laboratory Safety Manual

FBI Laboratory Quality Assurance Manual

FBI Laboratory Operations Manual

DNA Procedures Manual

Rev. #	Issue Date	History
0	02/05/16	Reformatted from Mitochondrial DNA Analysis Laboratory Procedures. Added ability to use procedure on FNC. Added option to add additional reagent and time for digesting. Increased elution volume to 65. Added option to grind if full digestion is not achieved.

Approval

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